Exhibit 4



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## WW domains of Nedd4 bind to the proline-rich PY motifs in the epithelial Na+ channel deleted in Liddle's syndrome.

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The amiloride-sensitive epithelial sodium channel (ENaC) plays a major role in sodium transport in kidney and other epithelia, and in regulating blood pressure. The channel is composed of three subunits (alphabetagamma) each containing two proline-rich sequences (P1 and P2) at its C-terminus. The P2 regions in human beta and gammaENaC, identical to the rat betagammarENaC, were recently shown to be deleted in patients with Liddle's syndrome (a hereditary form of hypertension), leading to hyperactivation of the channel. Using a yeast two-hybrid screen, we have now identified the rat homologue of Nedd4 (rNedd4) as the binding partner for the P2 regions of beta and gammarENaC. rNedd4 contains a Ca2+ lipid binding (CaLB or C2) domain, three WW domains and a ubiquitin ligase (Hect) domain. Our yeast two-hybrid and in vitro binding studies revealed that the rNedd4-WW domains mediate this association by binding to the P2 regions, which include the PY motifs (XPPXY) of either betarENaC (PPPNY) or gammarENaC (PPPRY). SH3 domains were unable to bind these sequences. Moreover, mutations to Ala of Pro616 or Tyr618 within the betarENaC P2 sequence (to PPANY or PPPNA, respectively), recently described in Liddle's patients, led to abrogation of rNedd4-WW binding. Nedd4-WW domains also bound to the proline-rich C-terminus (containing the sequence PPPAY) of alpharENaC, and endogenous Nedd4 co-immunoprecipitated with alpharENaC expressed in MDCK cells. These results demonstrate that the WW domains of rNedd4 bind to the PY motifs deleted from beta or gammaENaC in Liddle's syndrome patients, and suggest that Nedd4 may be a regulator (suppressor) of the epithelial Na+ channel.

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